APPENDIX

Photographic Grading and Definitions of AMD (sub-section)

Detailed description of the steps of the Three Continent Consortium (3CC) 5-step severity scale of age-related macular degeneration:

Level	Label	Description
10	No AMD	No, questionable, small or intermediate sized drusen (<125µm in diameter) only, regardless of area of involvement and no pigmentary abnormality (defined as increased pigment or RPE depigmentation present); or No definite drusen with any pigmentary abnormality.
20	Minimally severe early AMD	Small to intermediate sized drusen (<125µm in diameter), regardless of area of involvment, with any pigmentary abnormality; or Large drusen (≥125µm in diameter with drusen area <331,820µm^2 (equivalent to the Wisconsin AMD grading circle O-2, defined as a circle with diameter of 650µm) and no pigmentary abnormalities.
30	Moderately severe early AMD	Large drusen (≥125µm in diameter) with drusen area <331,820µm^2 and with any pigmentary abnormality; or Large drusen (≥125µm in diameter) with drusen area ≥331,820µm^2 with or without increased retinal pigment but no RPE depigmentation.
40	Severe early AMD	Large drusen (≥125µm in diameter) with drusen area ≥331,820µm^2 and RPE depigmentation present, with or without increased retinal pigment.
50	Late AMD	Pure geographic atrophy in the absence of exudative macular degeneration; or Exudative macular degeneration with or without geographic atrophy present.
AMD=age-related macular degeneration; RPE=retinal pigment epithelium		

Genotyping Methods

In the BMES genotyping was performed using a custom array (Human 670-Quad, version 1; Illumina Inc) at the Wellcome Trust Centre for Human Genetics, Sanger Institute, Cambridge, UK, as part of the Wellcome Trust Case Control Consortium 2[1]. After quality-control checking, genotypes of 2534 participants (544, 802 single nucleotide polymorphisms (SNPs)) were imputed from a genetic variation catalogue (1000 Genomes, version 1) using IMPUTE software (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html), accessed June 4, 2013). Additionally, genotype data was previously obtained for *CFH* SNP rs1061170 in 1840 participants and *ARMS2* SNP rs10490924 in 615 participants who attended the 5-year follow-up[2], and these subjects had one or both of the SNPs typed and imputed. The concordance rates between typed and imputed SNPs were 99.6% for rs1061170 and 99.2% for rs10490924. The genotyped SNPs were used whenever available.

In the BDES, *CFH*-rs1061170 was genotyped using TaqMan assays (Applied Biosystems, Foster City, CA) in 3015 participants, and a custom Illumina array in 2940 participants with subsequent data imputation techniques performed with Markov chain haplotyping, version 1.0.32 (http://www.sph.umich.edu/csg/abecasis/MACH/)[3]. The concordance rate between typed and imputed data among 1476 samples was 99.8%. Similarly the *ARMS2*-rs10490924 SNP was genotyped using 2 platforms, including TaqMan assay and an iSelect array

(Illumina, Inc.). The concordance rate between the 588 samples genotyped with both platforms was 99.7%[3].

In the RS, *CFH*-rs1061170 was successfully genotyped in 6345 participants and *ARMS2*-rs10490924 in 6411, using TaqMan assays (Applied Biosystems, Foster City, CA)[4]. Additionally, for participants without genotyped data, data was imputed from a genome-wide association scan dataset, genotyped using the Illumina Infinium II HumanHap550[4]. Imputation was performed using Markov Chain Haplotyping software version 1.0.15 (http://www.sph.umich.edu/csg/abecasis/MACH/, accessed June 4, 2013) and HapMap CEU data (NCBI build 36, release 22, The International HapMap Project). There were 6478 participants with typed or imputed *CFH* and *ARMS2* SNPs.

References:

1. Holliday EG, Smith AV, Cornes BK, et al. Insights into the Genetic Architecture of Early Stage Age-Related Macular Degeneration: A Genome-Wide Association Study Meta-Analysis. PLoS ONE 2013;8:e53830.

2. Wang JJ, Rochtchina E, Smith W, et al. Combined effects of complement factor H genotypes, fish consumption, and inflammatory markers on long-term risk for age-related macular degeneration in a cohort. Am J Epidemiol 2009;169:633-41.

3. Klein R, Myers CE, Meuer SM, et al. Risk alleles in CFH and ARMS2 and the long-term natural history of age-related macular degeneration: the Beaver Dam Eye Study. JAMA Ophthalmol 2013;131:383-92.

4. Buitendijk GH, Rochtchina E, Myers C, et al. Prediction of age-related macular degeneration in the general population: the Three Continent AMD Consortium. Ophthalmology 2013;120:2644-55.